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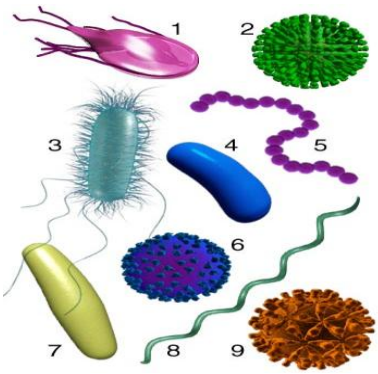

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2010-2011 1431-1432

Lecture 3

Bacterial physiology and metabolism

The physiology and metabolic activities of bacteria are widely divergent. The aim of these activities is growth and reproduction

Bacterial growth

It is increase in number of bacterial cells rather than increase in size of individual bacteria.

Bacterial species only maintained if population continues to grow Microbial growth = increase in number of cells, not cell size

Bacterial cell is able to duplicate itself by binary fission which involves many chemical reactions

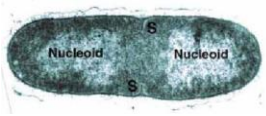
•– Energy transformation

•– Biosynthesis of small molecules (enzymes, cofactors)

•– Polymerisation of macromolecules from monomers

• Assembly of macromolecules, formation of cellular structures

•(cell wall, cytoplasmic membrane …….)



Binary fission

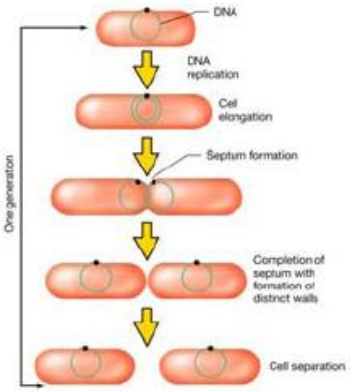
Cells elongate to approx 2x length of smallest cell

– Partition (septum) forms that eventually divides the cell into 2 daughter cells;

– Two daughter cells are pinched off During growth cycle all cellular components increase in number so each daughter cell receives a complete chromosome, macromolecules

• Note that DNA remains attached to cell membrane during division & replication of the chromosome so that as septum forms each daughter cell receives a complete chromosome copy

• Time required for a complete growth cycle is very variable dependent on nutritional & genetic factors



Population growth

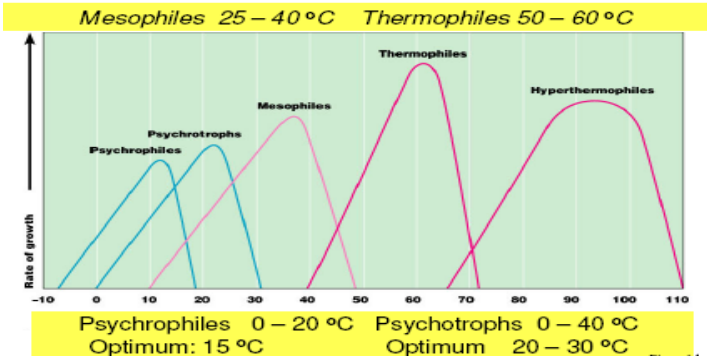
Growth rate = change in cell number or cell mass it time.

• Generation = formation of 2 daughter cells from one cell
• Generation time = time for one generation to occur; generation time = doubling time

• Note during one generation both cell number and cell mass double

Bacterium	Mean generation time (hours)
Bacillus megaterium	0.5
Escherichia coli	0.33
Treponema pallidum	34
*Saccharomyces cerevisiae** (a fungus)	2
*Paramecium caudatum** (a protozoa)	10.5

Requirements for Bacterial Growth:



Physical Requirements
I-Temperature
Range min. to max.
Minimum growth temperature
Optimum growth temperature “ideal”
Maximum growth temperature

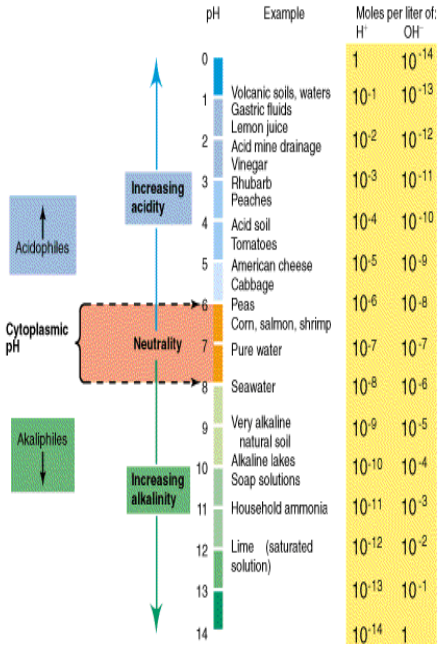
2-pH

Most bacteria grow between pH 6.5 and 7.5, **Neutrophiles**

Molds and yeasts grow between pH 5 and 6

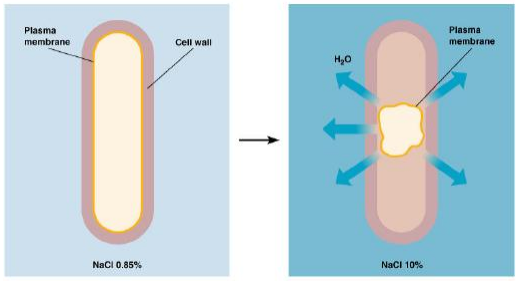
Acidophiles grow in acidic environments while

Alkalophiles grow in alkaline environments



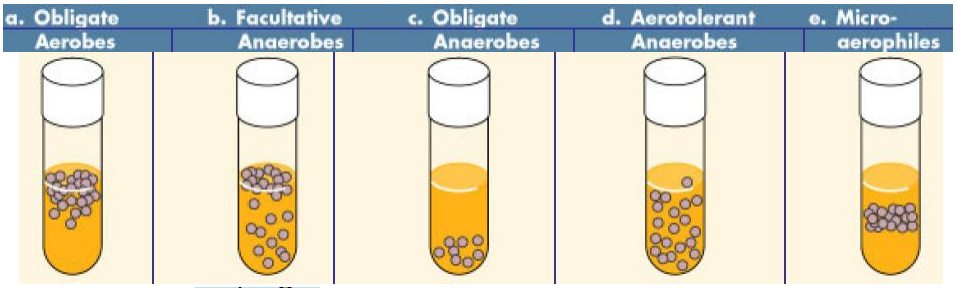
3-Osmotic Pressure

- Hypertonic environments, increase salt or sugar, cause plasmolysis
- Extreme or obligate halophiles *require* high osmotic pressure
- Facultative halophiles *tolerate* high osmotic pressure



II- Chemical requirements :

1-Oxygen Some organisms need oxygen for its growth while Other cannot live if it is present and some will tolerate

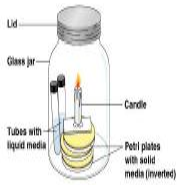


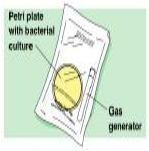
Can grow …..

- Candle jar

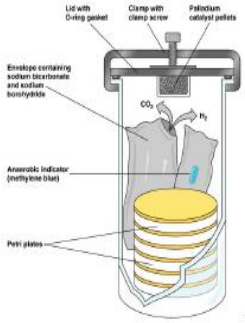
Resemble conditions in intestinal tract

- CO_2-packet

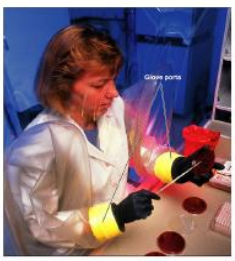




- Anaerobic jar
- For removing Oxygen; replaced with CO_2



- Anaerobic chamber -
- Is filled with oxygen-free gas
- eg. CO_2



II- Chemical requirements :

2-Carbon,
• Heterotrophs use organic carbon sources (sugars and proteins)
• Autotrophs use simple inorganic source (CO_2) and nitrogen (amm.sulfate). The energy needed by these bacteria is obtained either by light or from the oxidation of inorganic substances
3-Nitrogen
• In amino acids, proteins. • Most bacteria decompose proteins
• Some bacteria use NH_4 or NO_3
•A few bacteria use **N_2** in “nitrogen fixation” • take atmospheric nitrogen and form compounds. in legumes
4-Sulfur
• In amino acids, thiamine, biotin. Most bacteria decompose proteins
• Some bacteria use **SO_4** or **H_2S** & a.a. with sulfur • e.g. (Cys forms cross links)
5- Phosphorus
• In DNA, RNA, ATP, and membranes. • PO_4^{-2} is a source of phosphorus

6-Trace Elements
• Inorganic elements required in small amounts
• Usually as enzyme cofactors • eg. copper, iron, magnesium

Bacterial Nutrition:

According to the nutritional requirements bacteria are classified into:

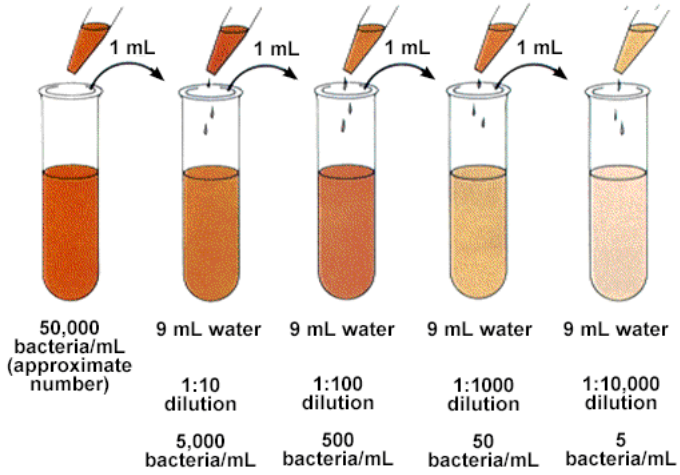
1- **Autotrophic** bacteria use simple inorganic source (CO_2) and nitrogen (amm.sulfate). The energy needed by these bacteria is obtained either by light or from the oxidation of inorganic substances

2- **Heterotrophic** bacteria use organic carbon sources (sugars and proteins)

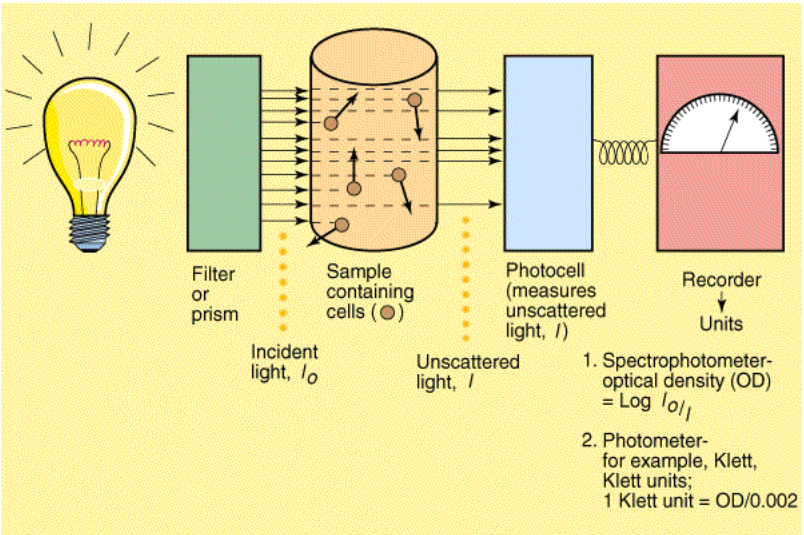
Measuring of bacterial Growth

- Direct Counts
  - Petroff-Hauser Chamber
- Serial Dilution
  - 10-fold serial dilutions
- Turbidity
  - Spectrophotometer
  - Scale
    - %Transmittance
    - Optical Density or Absorbance
- Filtration
  - 0.45 - 0.2 um sizes
  - Grid Pattern on Filter
  - Standards for Public Health
    - 0 E.coli / 100 ml of water
  - Also used for sterilization

1-Serial dilution method



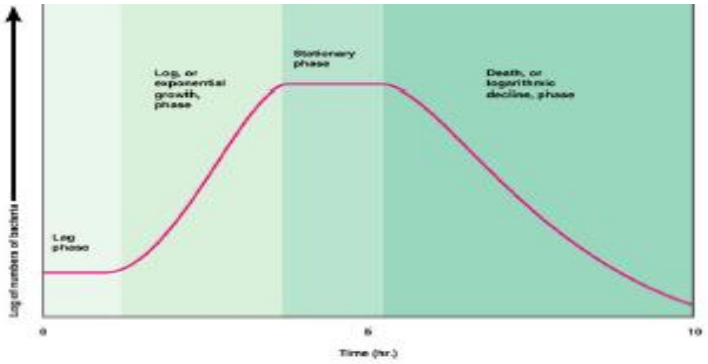
2-Turbidity method using spectrophotometer



Bacterial growth curve

several distinct phases can be seen in the bacterial growth curve:

– Lag phase – Exponential growth – Stationary phase Death phase



– Note:
this describes phases of bacterial growth of a population of cells, not individual cells

Lag phase:

When microbes inoculated into fresh medium they do not start to grow immediately (lag phase).
Length of lag phase variable depends on history of the culture and growth conditions

1-exponentially growing culture inoculated into same media,same growth conditions no lag phase.
2-old culture, there is a lag phase because cells need to replenish essential constituents to start growth & cell division cycle.
3-Cells damaged (heat, radiation, toxic chemicals) , there is a lag phase as cells repair damage.
4-Cells transferred from rich medium to poor culture medium, lag phase as cells have to synthesize more enzymes etc to enable synthesis of macromolecules not present in poor culture medium.

Exponential phase

• Each cell divides to form 2 cells; 2 cells divide to form 4 cells
• Rate of exponential growth influenced by environmental conditions (temperature, composition of culture medium) & genetic characteristics of organism.

Stationary phase:

In a batch culture exponential growth cannot occur indefinitely
– Essential nutrients in medium used up and/or
– Some waste product of the organism builds up to an inhibitory advice
– Exponential growth ceases = stationary phase
• In stationary phase – no net increase or decrease in cell number

Death phase

If incubation continues after stationary phase, cells may remain alive and continue to metabolise OR they may die = death phase
• In some cases cell death is accompanied by lysis
• Rate of cell death generally slower than that of exponential growth

Culture Medium: Nutrients prepared for microbial growth
Sterile: No living microbes
Inoculum: Introduction of microbes into medium
Culture: Microbes growing in/on culture medium

Reducing media:Contain chemicals (thioglycolate) that combine With Heated to drive off O_2
Selective Media: Suppress unwanted microbes and encourage desired microbes.
e.g, EMB medium both selective and differential (Selects for Gram - and differentiates between coloiform bacteria eg. *E.coli*. Gram + growth is inhibited) so is also selective.

Differential Media: Make it easy to distinguish colonies between different kinds of microbes. (e.g. **Eosin Methylene Blue (**EMB), Blood agar,
TSA medium Tryptic Soy Agar: differentiates between species of Streptococcus

Preserving Bacteria Cultures:

Deep-freezing: -50°to -95°C Often suspended in 50% glycerol
Lyophilization (freeze-drying): Frozen (-54°to -72°C) and dehydrated in a vacuum

Thanks!